AF/DW

Atty. Dkt. No. 076333-0325

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Louis D. Falo et al.
Title:	INDUCTION OF TUMOR AND VIRAL IMMUNITY USING ANTIGEN PRESENTING CELL CO-CULTURE PRODUCTS AND FUSION PRODUCTS
Appl. No.:	10/608,424
Filing Date:	06/30/2003
Examiner:	Gerald R. Ewoldt
Art Unit:	1644
Confirmation Number:	8081

BRIEF ON APPEAL TRANSMITTAL

Mail Stop Appeal Brief - Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Transmitted herewith is an appeal brief in the above-identified application:

Submitted herewith in connection with the above application are the following:

[X] Response to Notification of Non-Compliant Appeal Brief

[X] Evidence Appendix (containing 1 reference).

[X] Related Proceedings Appendix (containing 1 attachment).

[X] Power of Attorney from parent application (4 pgs.).

[X] A credit card payment form in the amount of $775.00 is enclosed in payment of fee for filing a brief in support of an appeal under 37 CFR 1.17(c).

[X] Applicant hereby petitions for an extension of time under 37 C.F.R. §1.136(a) for the total number of months checked below:

12/27/2007 AWONDAF1 00000054 10608424
01 FC:2253 525.00 OP

~~12/27/2007 AWONDAF1 00000054 190741 10608424~~
~~02 FC:2402 5.00 DA 250.00 OP~~

Atty. Dkt. No. 076333-0325

[X]	Extension for response filed within the third month:	$1,050.00	$1,050.00
[X]	Appeal Brief	$500.00	$500.00
	CLAIMS, EXTENSION AND DISCLAIMER FEE TOTAL:		$1,550.00
[X]	Small Entity Fees Apply (subtract ½ of above):		$775.00
	TOTAL FEE:		$775.00

A credit card payment form in the amount of $775.00 is enclosed.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by the credit card payment form being unsigned, providing incorrect information resulting in a rejected credit card transaction, or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741.

Please direct all correspondence to the undersigned attorney or agent at the address indicated below.

Respectfully submitted,

Date December 26, 2007

By S. A. Bent

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Atty. Dkt. No. 076333-0325

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Applicant:	Louis D. Falo *et al.*
Title:	INDUCTION OF TUMOR AND VIRAL IMMUNITY USING ANTIGEN PRESENTING CELL CO-CULTURE PRODUCTS AND FUSION PRODUCTS
Appl. No.:	10/608,424
Filing Date:	6/30/2003
Examiner:	Gerald R. Ewoldt
Art Unit:	1644
Confirmation Number:	8081

BRIEF ON APPEAL AND RESPONSE TO NOTICE OF NON-COMPLIANT APPEAL BRIEF

Mail Stop Appeal Brief - Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This appeal brief was originally filed on January 25, 2007, along with the $250.00 appeal fee prescribed by 37 C.F.R. 41.20(b)(2) and a Petition for Extension of Time. In response, the Office issued a Notification of Non-Compliant Appeal Brief dated March 30, 2007. Although Appellants believe that the original appeal brief was fully compliant, Appellants filed another appeal brief on April 30, 2007, which addressed the issues raised in the March 30th Notification.

Applicants received yet another Notification of Non-Compliant Appeal Brief on August 24, 2007. The August 24th Notification alleged that "[t]he Evidence Appendix does not state where Maranon et al. was entered into the record by the Examiner as is required." However, the Evidence Appendices in both appeal briefs state that "Marañón was cited in Appellants' response of November 7, 2005 at page 6." More specifically, Marañón was cited

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in response to a non-final Office Action to rebut a factual assertion made by the examiner. As such, MPEP § 609.05(c) compels its consideration. *See* MPEP § 609.05(c) ("[t]o the extent that a document is submitted as evidence directed to an issue of patentability raised in an Office action, and the evidence is timely presented, applicant need not satisfy the requirements of 37 CFR 1.97 and 37 CFR 1.98 in order to have the examiner consider the information contained in the document relied on by applicant"). Thus, both appeal briefs fully satisfied the requirements of the Evidence Appendix.

Applicants representative attempted to contact Examiner Ewoldt multiple times by telephone to resolve this issue, but Examiner Ewoldt never returned any of the telephone calls leaving Applicants no choice but to file this appeal brief yet again. Because the Evidence Appendix, in this appeal brief, the January 25th appeal brief, and the April 30th appeal brief, satisfies the requirements of 37 C.F.R. 41.37(ix).

Finally, Applicants have updated the Related Proceedings Appendix, because a decision has issued in the related application.

Appellants enclose a Petition for Extension of Time to make this paper timely. If any additional fees necessary to timely file this appeal brief are deemed insufficient, authorization is hereby given to charge any deficiency (or credit any balance) to the undersigned deposit account 19-0741.

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REAL PARTY IN INTEREST

The real party in interest is the University of Pittsburgh, the assignee of this application.

RELATED APPEALS AND INTERFERENCES

As to an appeal or interference that may directly affect, be directly affected by, or have a bearing on the Board's decision in present appeal, Appellants are aware only of an appeal pending in relation to an application, serial No. 09/208,549 (Atty. Dkt. No. 076333-0242), that is related, as a divisional, to the above-captioned application. The issues presented in the appeal of the '549 application are entirely distinct from those of the present appeal.

STATUS OF CLAIMS

Claims 1, 2, and 4-12 are pending and subject to examination on the merits. Claims 3 and 13-26 were canceled previously.

STATUS OF AMENDMENTS

Appellants made no amendments after the Final Office Action mailed January 26, 2006. All amendments have been entered.

SUMMARY OF CLAIMED SUBJECT MATTER

Two independent claims, claims 1 and 8, are on appeal. Applicants provide below "a concise explanation of the subject matter defined in each of the independent claims involved in the appeal, referring to the specification by page and line number." *See* 37 C.F.R. § 41.37.

Claim 1 relates to a formulation comprising at least one hybridoma that is the fusion of at least one antigen presenting cell and at least one virally infected cell. *See* specification at page 3, line 30 – page 4, line 4; page 4, lines 10-15; page 6, lines 9 and 10; page 8, lines 3-6. The antigen presenting cell can be a macrophage or a dendritic cell. *Id.* at page 7, lines 15-

17. The specification explains that these formulations can be used to treat viral infection by inducing cytotoxic T-lymphocytes (CTLs). *Id.* at page 4, lines 5-22.

Claim 8 further relates to a pharmaceutical composition comprising a hybridoma, as claimed in claim 1, and a suitable pharmaceutical carrier. *Id.* at page 3, line 30 – page 4, line 4; page 4, lines 10-12; page 6, lines 9 and 10; page 8, line 32 – page 9, line 6. The specification explains that these formulations can be used to treat viral infection by inducing cytotoxic T-lymphocytes (CTLs). *Id.* at page 4, lines 5-22.

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

This appeal presents a single ground of rejection for review.[1] Specifically, Appellants present for consideration the rejection of claims 1, 2, and 4-12 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement.

ARGUMENT

The specification contains a complete description of the claimed invention, sufficient to allow one skilled in the relevant art to make and use the claimed invention without undue experimentation, as evinced by the working examples and corroborative literature. The Examiner's arguments to the contrary are critically flawed.

The examiner argues that the specification fails to enable how to *make* claimed invention. This argument fails because it is premised on the wrong standard for determining enablement. Specifically, the examiner imposes the requirement that the hybridoma be "immortal." Yet, the "invention that one skilled in the art must be enabled to make and use is

[1] The Final Office Action also provisionally rejected claims 1, 2, and 4-12 over claims 1, 2, and 4-12 of co-pending serial No. 11/089,035 (Atty. Dkt. No. 076333-0366). Appellants need not address this rejection here, however, because of its provisional nature.

that defined by the claim(s)," and the appealed claims do not require the "hybridoma" to be "immortal." MPEP § 2164.

The examiner also argues that the specification fails to enable a skilled artisan to *use* the claimed invention, because the invention allegedly cannot be used to treat HIV infection without undue experimentation. This argument fails for two separate reasons. First, the examiner improperly has discounted evidence proving that the claimed invention is enabled. Thus, the examiner has failed to consider the evidence as a whole, as he is required to do. *In re Wands*, 858 F.2d at 731, 737, 8 USPQ2d 1400, 1407. Second, the examiner has failed to demonstrate that the entire claim scope is enabled. Instead, the examiner focuses on the alleged lack of enablement of a single embodiment, curing HIV. Even were the single embodiment not enabled, as the examiner contends, it still would be the case that the "presence of inoperative embodiments within the scope of a claim does not necessarily render a claim nonenabled." MPEP § 2164.08(b); *see also Capon v. Eshhar*, 418 F.3d 1349, 1359, 76 USPQ.2d 1078, 1085 (Fed. Cir. 2005); *In re Angstadt*, 537 F.2d 498, 503, 190 USPQ 214, 218 (CCPA 1976). Here, there is no evidence or explanation that it would require undue experimentation to identify any inoperative embodiments.

A. The Specification Enables One Of Skill In the Art To Make The Claimed Invention (Claims 1, 2, and 4-12)

The specification contains sufficient guidance to make the claimed invention without undue experimentation. The claims are drawn to a formulation and pharmaceutical composition comprising "at least one hybridoma having at least one first cell fused to at least one second cell." The first cell is an antigen presenting cell (APC) selected from a macrophage and a dendritic cell (DC), and the second cell is a "virally infected cell." The specification teaches that "the hybridomas ... of the present invention can be formed by any

method known in the art." Specification at page 8, lines 3-4. Indeed, methods were well-known in the art for fusing antigen presenting cells, such as dendritic cells and macorphages, to other cells. *See Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986) (noting that a specification preferably omits what is known in the art). The specification goes on to provide a method for forming a hybridoma from an APC and virally infected cell, using polyethylene glycol (PEG). *Id.* at page 8, lines 4-11. Moreover, the specification contains working examples demonstrating the formation of a hybridoma. *Id.* at page 11, lines 5-19. Thus, the specification provides guidance as to how to construct the claimed formulation.

The Examiner does not dispute that the recited APCs can be fused with a "virally infected cell." Instead, he invokes literature citations to import a limitation into the claims, thereby to validate a conclusion of non-enablement. In particular, the Examiner relies on literature references to argue that a "hybridoma" is a cell line in "a state of unrestrained growth in culture, resembling or identical with the tumorigenic condition." Office Action at 3.[2] So saying, the Examiner purports to find in the present recitation of "hybridoma" a requirement for an "immortal" cell, and then judges enablement with reference to that requirement. *Id.*

But the claims do not require the hybridoma to be an immortal cell. "An applicant is entitled to be his or her own lexicographer," and "[w]here an explicit definition is provided by the applicant for a term, that definition will control interpretation of the term as it is used in the claim." MPEP § 2111.01(IV); *see Toro Co. v. White Consolidated Industries Inc.*, 199

[2] Unless otherwise states, citations to "Office Action" are to the Final Office Action dated January 26, 2006.

F.3d 1295, 1301, 53 USPQ2d 1065, 1069 (Fed. Cir. 1999) (meaning of words used in a claim is not construed in a "lexicographic vacuum, but in the context of the specification and drawings"). Here, the specification defines a hybridoma as "a physical combination of at least two different cell types." Specification at page 6, lines 14-15. In other words, the hybridoma is simply a fusion of at least two different cell types. The specification further specifies that the two different cell types can be "at least one APC and at least one virally infected cell." *Id.* at page 6, lines 15-18. Nothing in the specification requires the "hybridoma" to be an immortal cell. Indeed, the examiner does not rely on the specification to define a "hybridoma" as an immortal cell and instead resorts to literature references to import a requirement into the claims. Thus, it is improper to require enablement of an immortal "hybridoma" even assuming, *arguendo*, that one of skill in the art understands a "hybridoma" to be an immortal cell.

B. One Of Skill In The Art Could Use The Claimed Invention Without Undue Experimentation (Claims 1, 2, and 4-12)

"[W]hen a compound or composition claim is not limited by a recited use, any enabled use that would reasonably correlate with the entire scope of that claim is sufficient to preclude a rejection for nonenablement based on how to use." MPEP § 2165.02(c). "In other words, if any use is enabled when multiple uses are disclosed, the application is enabling for the claimed invention." *Id.* Here, the specification readily satisfies this standard.

The specification teaches that the claimed formulation and composition can "protect against the viral infection caused by the virally infected cells used in the formulation, and/or provide therapeutic relief from patients having such viral infections." Specification at page 4, lines 2-4. The specification explains that while virally infected cells can express antigens which can be targeted by cytotoxic T-lymphocytes (CTLs), the virally infected cells

themselves do not stimulate immunity. *Id.* at page 4, lines 5-7. This lack of immunity stems from the inability of virally infected cells to express the antigens in the appropriate context of co-stimulation. *Id.* at page 4, lines 7-9. The claimed invention overcomes this problem by fusing the virally infected cell to APCs, which express co-stimulatory molecules and cytokines. Because the fusions exhibit the properties of both the APC (expression of co-stimulatory molecules and cytokines) and the virally infected cell (expression of antigen), the fusions stimulate immunity and "result in the destruction of the virus." *Id.* at page 4, lines 17-22. The specification goes on describe in more detail how the claimed formulations and compositions can be used to treat viral infections. *See, e.g., id.* at pages 8-10. For instance, the specification teaches that the "effective amount" of the composition will vary depending on the patient and severity of the infection. Generally, however, the dosage will be about 1 x 10^6 cell equivalents to about 100 x 10^6 cell equivalents per treatment. *Id.* at page 10, lines 7-10. Thus, the specification teaches how the claimed invention is therapeutically useful by virtue of its ability to destroy virus.

The working examples corroborate the teachings of the specification and demonstrate that the claimed fusions are therapeutically useful. Indeed, the examples demonstrate that "immunization with products of [dendritic cell]-tumor cell fusions or co-cultures can induce tumor-specific CTLs and potent protective anti-tumor immunity against two distinct, poorly immunogenic tumors." Spec. at page 17, lines 3-6. In addition, the fusions were shown to eradicate existing tumors. *See* example 6 (page 17).

While the examples were directed to tumor cells rather than virally infected cells, there is no objective reason to doubt that the results would be similar for virally infected cells, because the same mechanism applies. Namely, the fusions combine the ability of APCs to

express co-stimulatory molecules and cytokines with the ability of tumor and virally infected cells to express antigen.

Indeed, a literature reference confirms the teachings of the specification also apply to virally infected cells. Specifically, Marañón *et al.*, *Proc. Nat'l Acad. Sci.* USA 101: 6092-97 (2004), studied the presentation of HIV antigens from dendritic cells and concluded that dendritic cells that present viral antigens stimulate virus-specific CD8+ cells. In fact, Marañón concluded that dendritic cell antigen presentation could be "exploited to eradicate latently infected reservoirs." Marañón, abstract (emphasis added). Thus, Marañón confirms that the claimed invention could be employed in the treatment of viral infections, including HIV infection.

The Examiner contends that undue experimentation is required to use the claimed invention, because the claimed formulations are "highly unpredictable" and "would be more likely to exacerbate viral infections than to treat or prevent them." Office action at 3. As support for this contention, the examiner cites publications generally relating to HIV: Frank, *Current Mol. Medicine* 2: 229 (2002); Cohen, *Science* 295: 1616 (2002); Roberts, *The Scientist* 18(11) (June 2004).

The Examiner's conclusion is flawed for at least two reasons, however. First, the Examiner improperly discounts evidence, Marañón, demonstrating that the claimed invention could be useful in treating viral infections, including HIV. Second, even if one assumes that the claimed invention is not enabled for treating HIV, claims 1-2 and 5-12 are not necessarily defective under Section 112, first paragraph, because "[t]he presence of inoperative embodiments within the scope of a claim does not necessarily render a claim nonenabled." MPEP § 2164.08(b).

1. Marañón Provides Evidence Of Enablement (Claims 1, 2, and 4-12)

Marañón demonstrates that DCs presenting HIV antigen could be "exploited to eradicate latently infected reservoirs," as discussed above. The examiner improperly dismisses Marañón.

At the outset, the examiner argues that Marañón is "not of record in this case and thus, need not be addressed." This is not correct. Marañón was first cited in Appellants' response of November 7, 2005, to the non-final Office Action dated June 7, 2005. Marañón was submitted to counter the Examiner's argument, presented in the June 7 Office Action, that the claims lack enablement. Thus, Marañón was timely submitted as evidence directed to an issue of patentability raised in the June 7th Office Action. Accordingly, MPEP § 609.05(c) compels its consideration by the Examiner.

The Examiner also argues that Marañón "cannot be used to establish the enablement of the instant application as of its priority date," because it was published "seven years after the priority date of the instant application." Office Action at 4. In addition, the examiner notes that Marañón "employs live antigen-loaded dendritic cells and not the fusion products of the instant claims" and that Marañón "addresses a number of issues that were clearly not known as of the priority date of the instant application." Office Action at 4.

These reasons for discounting Marañón are inapposite to Marañón's value in demonstrating the enablement of the claimed invention. It is well-established that a post-filing date reference can be used to demonstrate enablement when the reference merely demonstrates the state of the art as of the priority date. *See , e.g., Gould v. Quigg*, 822 F.2d 1074, 1078, 3 USPQ.2d 1302, 1305 (Fed. Cir. 1987). Marañón does precisely that,

employing the same general approach that Appellants described in their application and demonstrated successfully.

In challenging this proposition, the Examiner notes "issues that were clearly not known as of the priority date."[3] These "issues" had no bearing, when the invention was made, on what level of experimentation the skilled person needed to implement Appellants' teachings in the present specification. For instance, the Examiner argues that one of the allegedly unknown issues was "the manner in which dendritic cells take up and present antigens." Office Action at 4. Yet, one of skill in the art would not need to understand the mechanism of antigen presentation in order practice the claimed invention. In other words, operational sufficiency does not require an understanding of the theoretical underpinnings of the operation.

Finally, the fact that Marañón used antigen-loaded dendritic cells, rather than fusion products, in no way detracts from the probity of Marañón in vindicating the enabled quality of the appealed claims. Simply put, the Examiner has proffered no evidence or explanation why the results obtained from using fusion products would differ from results obtained using Marañón's co-culture cells. Thus, the Examiner has failed to satisfy his evidentiary burden. Moreover, the Examiner cannot satisfy his evidentiary burden on this point, because Appellants' working examples demonstrate the success of both fusion and co-culture techniques. Thus, Appellants' working examples directly contradict the Examiner's efforts to discount Marañón. Accordingly, the examiner improperly dismissed Marañón in rejecting the present claims as allegedly lacking enablement.

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[3] While the Examiner contends that Marañón addresses "issues that were clearly not known as of the priority date," the Examiner identified only a single issue, the mechanism by which DCs "take up and present antigens." Office Action at 4.

2. The Examiner's Arguments Fail To Demonstrate The Enablement Of The Entire Claim Scope (Claims 1-2 And 5-12)

Even if taken as true, the examiner's arguments fail to satisfy the burden in establishing a rejection of lack of enablement for claims 1-2 and 5-12. The examiner focuses almost exclusively on HIV with some passing reference to other viruses, but claims 1-2 and 5-12 are not directly exclusively to HIV. Instead, they generally cover "virally infected cells." Thus, at best, the examiner has identified only a single inoperative embodiment. Such a showing is not sufficient to reject the claims as lacking enablement, because "[i]t is not necessary that every permutation within a generally operable invention be effective in order for an inventor to obtain a generic claim, provided that the effect is sufficiently demonstrated to characterize a generic invention." *Capon v. Eshhar*, 418 F.3d 1349, 1359, 76 USPQ.2d 1078, 1085 (Fed. Cir. 2005); *see also Application of Cook*, 439 F.2d 730, 735, 169 USPQ 298, 302 (CCPA 1071) (holding that claims reading on "very large number of inoperative embodiments" enabled because inoperative embodiments could be readily identified); *In re Angstadt*, 537 F.2d 498, 503, 190 USPQ 214, 218 (CCPA 1976); MPEP 2164.08(b). Thus, even if one assumes, *arguendo*, that the specification fails to enable treatment of HIV using the claimed formulation, such a showing does not demonstrate that the claims lack enablement.

C. Conclusion

Appellants respectfully request that the rejection of claims 1, 2, and 4-12 be reversed, because a proper analysis of the evidence as a whole fails to establish that the claims lack enablement.

Respectfully submitted,

Date December 26, 2007

By 

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CLAIMS APPENDIX

1. (Previously Presented) A formulation comprising at least one hybridoma having at least one first cell fused to at least one second cell; wherein said first cell is an antigen presenting cell selected from the group consisting of a macrophage and a dendritic cell, and said second cell is a virally infected cell.

2. (Previously Presented) The formulation of claim 1, wherein said dendritic cells are selected from the group consisting of cutaneous epidermal Langerhans cells, dermal dendritic cells, lymph node dendritic cells, spleen dendritic cells and dendritic cells derived through in vitro culture of precursors.

3. (Canceled).

4. (Previously Presented) The formulation of claim 1, wherein said virally infected cells are selected from the group consisting of cells infected with influenza virus, human immunodeficiency virus, cytomegalovirus, human papilloma virus and herpes simplex virus.

5. (Previously Presented) The formulation of claim 1, wherein said hybridoma contains a ratio of first cells to second cells between about 1:100 and 100:1.

6. (Previously Presented) The formulation of claim 1, wherein said hybridoma contains a ratio of first cells to second cells of between about 1:10 and 10:1.

7. (Previously Presented) The formulation of claim 1, wherein said hybridoma contains a ratio of first cells to second cells of about 6:1.

8. (Previously Presented) A pharmaceutical composition comprising: at least one hybridoma; and a suitable pharmaceutical carrier; wherein each hybridoma is comprised of at least one first cell fused to at least one second cell; wherein said first cell is

an antigen presenting cell selected from the group consisting of a macrophage and a dendritic cell, and said second cell is a virally infected cell.

9. (Previously Presented) The pharmaceutical composition of claim 8, wherein said suitable pharmaceutical carrier is selected from the group consisting of saline and phosphate buffered saline.

10. (Previously Presented) The pharmaceutical composition of claim 8, wherein said hybridomas have a ratio of first cells to second cells of between about 1:100 and 100:1.

11. (Previously Presented) The pharmaceutical composition of claim 8, wherein said hybridomas have a ratio of first cells to second cells of between about 1:10 and 10:1.

12. (Previously Presented) The pharmaceutical composition of claim 9, wherein said hybridomas have a ratio of first cells to second cells of about 6:1.

13.-36. (Canceled).

EVIDENCE APPENDIX

Marañón *et al.*, PNAS 101(16):6092:97 (2004) – Marañón was cited in Appellants' response of November 7, 2005 at page 6.[4] A copy of Marañón is attached.

[4] MPEP § 609.05(c) ("[t]o the extent that a document is submitted as evidence directed to an issue of patentability raised in an Office action, and the evidence is timely presented, applicant need not satisfy the requirements of 37 CFR 1.97 and 37 CFR 1.98 in order to have the examiner consider the information contained in the document relied on by applicant").

RELATED PROCEEDINGS APPENDIX

A decision issued in Appl. No. 09/208,549 (Atty. Dkt. No. 076333-0242) on July 12, 2007, a copy of which is attached.

Dendritic cells cross-present HIV antigens from live as well as apoptotic infected CD4$^+$ T lymphocytes

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Edited by Philippa Marrack, National Jewish Medical and Research Center, Denver, CO, and approved February 24, 2004 (received for review August 1, 2003)

A better understanding of the antigen presentation pathways that lead to CD8$^+$ T cell recognition of HIV epitopes *in vivo* is needed to achieve better immune control of HIV replication. Here, we show that cross-presentation of very small amounts of HIV proteins from apoptotic infected CD4$^+$ T lymphocytes by dendritic cells to CD8$^+$ T cells is much more efficient than other known HIV presentation pathways, i.e., direct presentation of infectious virus or cross-presentation of defective virus. Unexpectedly, dendritic cells also take up actively antigens into endosomes from live infected CD4$^+$ T lymphocytes and cross-present them as efficiently as antigens derived from apoptotic infected cells. Moreover, live infected CD4$^+$ T cells costimulate cross-presenting dendritic cells in the process. Therefore, dendritic cells can present very small amounts of viral proteins from infected T cells either after apoptosis, which is frequent during HIV infection, or not. Thus, if HIV expression is transiently induced while costimulation is enhanced (for instance after IL-2 and IFNα immune therapy), this HIV antigen presentation pathway could be exploited to eradicate latently infected reservoirs, which are poorly recognized by patients' immune systems.

The prognosis of HIV infection has been greatly improved by highly active antiretroviral treatment. Efficient CD8$^+$ T cell responses are crucial for the development of a protective response against HIV (1). Specific CD8$^+$ T cells are detected during HIV primary infection, but their responses and phenotypes are altered compared with those found in other primary viral infections that are better controlled by the immune system (2, 3). Moreover, latently infected cells constitute viral reservoirs that are inaccessible to highly active antiretroviral treatment and do not reach the antigen expression threshold to stimulate directly HIV-specific CD8$^+$ T cells (4). To obtain better replication control, it would be important to obtain a potent and specific recognition of viral reservoirs by CD8$^+$ T cells.

HIV-specific CD8$^+$ T lymphocytes recognize viral peptides associated with MHC class I molecules on the surface of infected cells. They lyse these cells and produce IFNγ and other antiviral molecules. To proliferate and differentiate into effector cells, naive CD8$^+$ T lymphocytes require antigen presentation by dendritic cells (DC) (5). DC are infrequently infected by the virus as compared with CD4$^+$ T lymphocytes (6). Productive infection may therefore not be the only source of antigen for DC to induce HIV-specific CD8$^+$ T cell responses.

An attractive potential source of HIV antigens *in vivo* may be the apoptotic infected CD4$^+$ T lymphocytes typically induced by the infection (7). Apoptotic cells are targeted to specific receptors on macrophages and DC, which phagocytose them (8). DC have developed specific cross-presentation pathways that allow MHC class I-restricted presentation of the antigens contained in these apoptotic cells to CD8$^+$ T lymphocytes (9, 10). DC from HIV$^+$ patients can activate autologous CD4$^+$ and CD8$^+$ lymphocytes after coculture with infected apoptotic cells (11, 12). An alternative source of HIV antigens for DC may be defective viral particles, which can fuse with the plasma membrane and be cross-presented without viral replication (13). The relative importance of these mechanisms for HIV presentation has never been evaluated.

We have compared these mechanisms on a quantitative basis to assess those that would be relevant *in vivo* in infected patients. We found that apoptotic infected CD4$^+$ T cell cross-presentation by DC is much more efficient to present HIV antigens to specific CD8$^+$ T cell lines than direct infection or presentation of defective virus particles or proteins. Surprisingly, we found that DC cross-present equally well HIV antigens from live or apoptotic infected CD4$^+$ T lymphocytes, after active antigen acquisition. A good knowledge of the relative importance of these HIV presentation mechanisms in DC is crucial to stimulate them appropriately in infected patients, in order to help the immune system and control HIV replication.

Materials and Methods

Cell Culture. H9 and 8E5 cells were maintained in complete RPMI medium 1640 supplemented with 10% FCS. HIV protein expression was induced in 8E5 cells by phytohemagglutinin (PHA) (Murex Diagnostics, Chatillon, France) and phorbol 12-myristate 13-acetate (Sigma) for 5 days (14). Primary CD4$^+$ T blasts were obtained from healthy donor peripheral blood mononuclear cells (PBMC; Etablissement Français du Sang, Pitié Salpêtrière, Paris, according to ethical guidelines) after 3 days incubation in 1 μg/ml PHA and 10 units/ml IL-2 (Roche), then positive CD4 immunomagnetic selection (Miltenyi Biotec, Paris). HIV-specific CD8$^+$ T cell lines were generated by using PBMC of HIV$^+$ individuals from cohort studies with the approval of Cochin Hospital's ethics committee as described (15). DC were differentiated from elutriated monocytes from HLA-typed healthy donors for 5–7 days in granulocyte–macrophage colony-stimulating factor (GM-CSF; Schering–Plough) and IL-4 (PeproTech, London) (15). DC loading was performed in H-2000 medium (Stemcell Technologies, Neylan, France) with GM-CSF and IL-4.

Viruses, Peptides, and Antibodies. HIV-1_{lai} and an azidothymidine (AZT)-sensitive WT HIV-1 isolate from an antiretroviral naive patient were used (16). Replication and sensitivity to 200 μM AZT were checked by using CD4$^+$ T cell blasts alone or cocultured with DC, or CD4$^+$ T cell-loaded DC cocultured with CD8$^+$ T cells.

HIV-1 Nef_{73-82} (QVPLRPMTYK, HLA-A3-restricted), $RT_{476-484}$ (ILKEPVHGV, HLA-A2), Gag_{77-85} (SLYNTVATL,

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: AZT, azidothymidine; DC, dendritic cell(s); PBMC, peripheral blood mononuclear cells; LPS, lipopolysaccharide; Z-VAD, *N*-benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone; RT, reverse transcriptase; Ac-LDL, acetylated low density lipoprotein.

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www.pnas.org/cgi/doi/10.1073/pnas.0304860101

HLA-A2), Gag_{20-28} (RLRPGGKKK, HLA-A3), and human T-lymphotrophic virus-1 Tax_{11-19} (LLFGYPVYV, HLA-A2) were from Neosystem (Strasbourg, France). The following mAbs were used: anti-MHC class I W6/32 ascitis (1:100), anti-CD11c (pure or phycoerythrin-labeled) and anti-CD3-FITC (Becton Dickinson), and GaMIg-Cy5 (Caltag, South San Francisco, CA).

Cross-Presentation. H9 or 8E5 cells were irradiated (160 or 40 mJ/cm^2 at 312 nm, respectively), cultured for 6 h, and then cocultured with DC for 16 h. AZT (200 μM; Sigma) was added when specified. Primary CD4$^+$ T cell blasts were irradiated at 20 mJ/cm^2 24 h after infection (11), cultured for 6 h, and then cocultured overnignt with DC in AZT. After 1 h of culture, 1 μg/ml lipopolysaccharide (LPS) (*Escherichia coli*, Calbiochem) was added if required. Thereafter, DC were extensively washed, then used as antigen-presenting cells (20,000 per well) in a 6-h IFNγ enzyme-linked immunospot assay (15) using as effectors CD8$^+$ T cell lines or PBMC. CD4$^+$ T cells were depleted or CD8$^+$ T cells were enriched by using anti-CD4 beads or CD8 T cell isolation kit II, respectively (Miltenyi Biotec). Two or more effector:DC ratios were systematically tested in triplicate. DC class I haplotype was chosen according to CD8$^+$ cell line restriction. In some experiments, apoptotic cells were purified or excluded by using annexin-V-conjugated magnetic microbeads (Miltenyi Biotec), which were then separated by using 10 mM EDTA-PBS. Transwell plates were from Costar. *N*-benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone (Z-VAD-FMK) (Z-VAD, 10 μM, Sigma) was added to T cells for 6 h. HIV p24 was quantified in viral stocks or infected cell lysates by ELISA (Innogenetics).

DC Loading. T cells were labeled with PKH67 or PKH26 (Sigma) before irradiation. At the end of the DC/apoptotic cell coculture, annexin-V-APC (Bender MedSystems, Vienna) and CD11c labeling was performed. In competition assays, DC were incubated with 10 mg/ml mannan, 500 μg/ml polyG or 10 μg/ml RGD (Arg-Gly-Asp) (all from Sigma) for 40 min at 4°C before coculture with PKH67-labeled live or UV-irradiated H9-HIV cells. After 1 h, cells were washed and stained with anti-CD11c-phycoerythrin in presence of 5 mM EDTA. Cells were then fixed with 1% paraformaldehyde and analyzed by using a FACScalibur flow cytometer and CELLQUEST software (Beckton Dickinson). At least 10,000 viable DC were acquired. For microscopy purposes, cells were settled onto slides in mounting medium (DAKO) and coverslipped after paraformaldehyde fixation. In some experiments, transferrin-Alexa488 (10 μg/ml) or acetylated low density lipoprotein (AcLDL)-Alexa488 (10 μg/ml, Molecular Probes) were added during incubation to define the early and the late endosomal/lysosomal compartment, respectively. *In situ* Cell Death Detection Kit, TMR red was from Roche. Samples were acquired by using a TCS SP2 inverted confocal microscope (Leica Microsystems).

Results

DC Loaded with HIV-Infected Apoptotic T Cells Stimulate HIV-Specific CD8$^+$ Lines. To test cross-presentation of HIV antigens present in apoptotic T lymphocytes, the CD4$^+$ T lymphoma cell line H9 chronically infected by HIV-1_{lai} was UV-irradiated. Apoptotic cells were isolated by using annexin-V immunomagnetic separation and incubated with monocyte-derived DC from noninfected donors as described in *Materials and Methods*. As shown in Fig. 1*A*, an HLA-A2-restricted HIV-1 reverse transcriptase (RT)-specific T cell line recognized the epitope on the surface of DC. Other CD8$^+$ T cell lines specific for the HLA-A2- and HLA-A3-restricted Nef- and Gag-derived epitopes were also stimulated after presentation by DC loaded with apoptotic H9-HIV cells (Fig. 1 *B–D*). This recognition was restricted by MHC class I molecules because it was blocked by an anti-class I mAb, and only CD8$^+$ T cells secreted IFNγ, as assessed by intracellular cytokine flow cytometry (not shown). In addition, recognition depended on DC haplotype (Fig. 1*B*), excluding direct viral presentation by apoptotic H9-HIV cells.

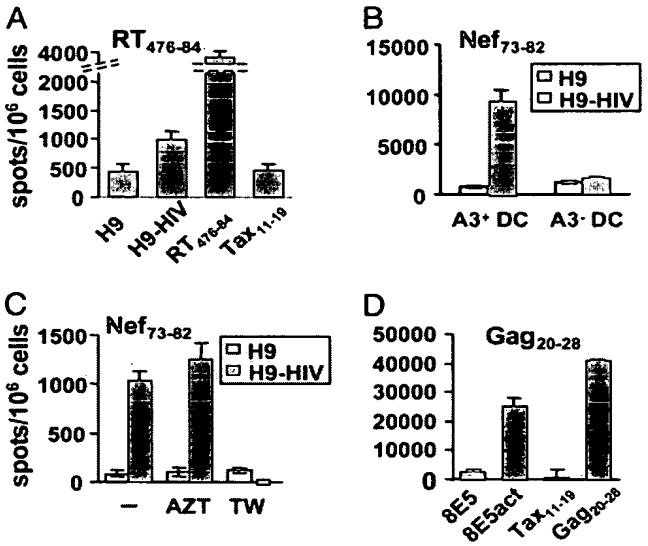

Fig. 1. DC presentation of MHC class I molecule-restricted viral epitopes from HIV-infected apoptotic CD4$^+$ T cells. (*A*) DC were incubated with apoptotic cells purified from irradiated H9 or H9-HIV cells at an H9:DC ratio of 3:1, then with LPS, and tested by using a CD8$^+$ T cell line specific for RT_{476-84} epitope at a DC:effector ratio of 1:3. As control, DC were incubated with 1 μM peptide. (*B*) The test was carried out by using DC expressing or not the class I MHC restriction molecule HLA-A3. (*C*) DC:H9 coculture was performed in the presence or not of AZT or in transwells (TW). (*D*) DC were cultured with irradiated 8E5 cells expressing viral proteins after activation (8E5act) in the absence of LPS and tested as before. Data are representative of at least three experiments each, except for *B* and *C* (two experiments).

Monocyte-derived DC do not constitute a preferential target for HIV, and only minor amounts of viral RNA are produced when they are infected *in vitro* (17, 18). Nevertheless, in our experimental system, DC were incubated overnight with HIV-producing cells; they could be infected and generate sufficient amounts of HIV epitopes to be presented to CD8$^+$ HIV-specific lines. To exclude direct viral presentation, DC and apoptotic cells were cocultured in the presence of AZT to block replication (19). DC presented HIV antigens in the absence of productive viral particle infection (Fig. 1*C*). Nonproductive infection of DC by defective viral particles can also yield antigen presentation to an HIV-specific CD8$^+$ clone (13). To investigate this potential mechanism, apoptotic cells were incubated in the upper compartment of a 0.45-μm transwell plate, allowing virion and soluble protein passage onto DC in the lower compartment, as measured by Bradford and Gag p24 ELISA tests, but preventing DC/apoptotic cell contact. In these conditions no specific response was detected (Fig. 1*C*), indicating that a contact or high proximity between DC and apoptotic cells was required for antigen uptake. These results suggested that viral infection, defective virion, or soluble antigen uptake are poorly efficient viral antigen acquisition mechanisms for MHC class I-restricted presentation by DC, compared with internalization of apoptotic debris. To study cross-presentation in the absence of infective virions, we used 8E5 cells, which are infected with an RT-defective HIV-1_{lai} variant and cannot produce infective viral particles, but produce low amounts of viral proteins after activation. The mean amount of p24 protein was 27 ± 36 ng of p24 per million cells for H9-HIV and 2 ± 1.5 pg of p24 per

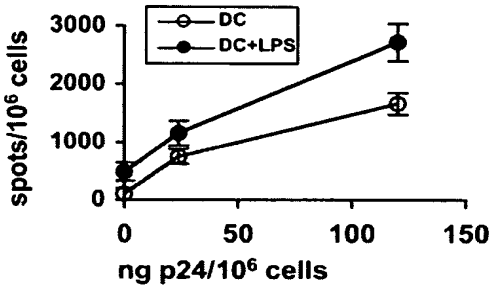

Fig. 2. DC cross-present a viral epitope from CD4+ primary lymphocytes infected with a WT HIV isolate. DC were cultured with irradiated CD4+ T cell blasts infected with different amounts of a primary HIV isolate in the presence or absence of LPS and tested by using an anti-Nef_{73-82} CD8+ line as in Fig. 1. This experiment was carried out in the presence of AZT.

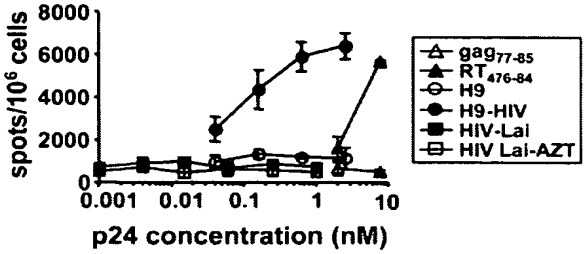

Fig. 3. Efficiency of cross-presentation of HIV antigens from apoptotic cells compared with free virus. DC were cultured with different amounts and sources of HIV epitope: synthetic peptides, UV-irradiated, apoptotic H9 and H9-HIV cells in the presence of AZT, or HIV-1-Lai with or without AZT; then LPS was added. After coculture, DC were tested by using an anti-RT_{476-84} CD8+ line. Equivalent Gag p24 values were calculated after titration of viruses or lysed cells. For synthetic peptides, molar concentrations are represented. Data are representative of two experiments.

million cells for activated 8E5 cells. Activated, irradiated 8E5 cells cocultured with DC induced efficient antigen presentation (Fig. 1*D*). These data show that DC actually cross-present MHC class I-restricted HIV antigens from apoptotic infected T cell lines to specific CD8+ T lymphocytes, in the absence of direct infection or of viral particles, even when low amounts of viral proteins are available.

Cross-Presentation of HIV Antigens from Apoptotic Primary CD4+ Lymphocytes Infected with a Wild-Type Virus Isolate. To investigate whether cross-presentation of chronically infected CD4+ lymphoid lines after apoptosis was transposable to primary T lymphocytes, CD4+ T cell blasts from an HLA-class I-mismatched donor were infected with different amounts of an AZT-sensitive primary HIV-1 isolate, irradiated, and incubated with DC before testing recognition by an HIV-specific CD8 T cell line. After overnight incubation of T cells or DC with 120 pg of p24 per 10^6 cells, p24 was detected in T cell lysates (28 ± 15 pg per million cells) but not in DC, where p24 production could be detected only after longer culture times, indicating poor infectability of DC compared with T cells (20). Nevertheless, to avoid any direct presentation by DC, DC-CD4+ T lymphocyte cocultures were performed with AZT. As seen in Fig. 2, loaded DC presented the Nef_{73-82} epitope to a specific CD8+ line proportionally to the amount of virus used to infect the CD4+ T blasts. Presentation was obtained even in the absence of LPS stimulation during DC-T cell coculture, although at a lower level than that in the presence of LPS. Indeed, CD4+ T cell blasts induced DC maturation (Fig. 6, which is published as supporting information on the PNAS web site). Therefore, DC cross-present HIV antigens from apoptotic primary CD4+ cells infected with WT HIV.

Cross-Presentation of HIV Antigens from Apoptotic T Cells Is More Efficient Than Direct Presentation or Presentation of Nonreplicating Virus. The different mechanisms of HIV antigen uptake and presentation mentioned above were compared quantitatively, by using p24 measurement in viral isolates or in apoptotic infected T cells before incubation with DC. Different amounts of $HIV\text{-}1_{lai}$, replication competent or not (without or with AZT), or apoptotic T cells (with AZT to avoid direct presentation) were incubated overnight with DC. As seen in Fig. 3, low amounts of p24 contained in apoptotic infected cells induced a half-maximal response around 0.1 nM, stronger than an equivalent molar concentration of the synthetic $RT_{476-484}$ peptide. Concentrations as high as 1 nM of p24 (equivalent to a multiplicity of infection of 200) of free virus, either infectious or inactivated by AZT, were not high enough to induce specific responses. When similar experiments were carried out by using activated 8E5 cells, even lower viral antigen concentrations (10^{-5} to 10^{-4} nM) induced CD8 T cell responses, even in the absence of LPS, probably due to more effective DC maturation in the presence of these cells (data not shown). Therefore, cross-presentation of HIV antigens from apoptotic infected CD4+ T cells was much more efficient on a quantitative basis than cross-presentation of defective virus or direct DC infection.

DC Acquire and Cross-Present Antigens from Live HIV-Infected T Cells. To test the dependence on apoptotic death of HIV cross-presentation, infected and non-infected H9 cells were labeled with the PKH67 cell membrane dye and cocultured with DC after being UV-irradiated or not. As shown in Fig. 4*A*, most of the DC (70–96% depending on the experiment) acquired PKH67 labeling, and neither the percentage of PKH67+ DC nor the fluorescence intensity was decreased if target cells were not previously irradiated. At 4°C, PKH67 acquisition was decreased to 19–24% of weakly positive DC, indicating that DC actively acquired material from live as well as apoptotic T cells (not shown). HIV-stimulated DC might have induced apoptosis in T lymphocytes, then phagocytosed the resulting debris (21). However, treatment of the cocultures with the pan-caspase inhibitor Z-VAD (22) did not reduce labeled material uptake (Fig. 4*A*). Apoptosis may also occur independently of caspases, but, in this experimental setting with low DC:H9 cell ratios, DC did not induce detectable apoptosis in nonirradiated cells anytime (Fig. 4 *B* and *C*; see also Fig. 4*D*, live). When live H9-HIV cells were cocultured with DC in the presence of Z-VAD, intimate DC-T contact and no T cell apoptosis were observed by confocal microscopy (Fig. 4*D*). All these data show that apoptosis induction is not required for antigen transfer. Material transfer from T lymphocytes into DC was visualized (Fig. 7, which is published as supporting information on the PNAS web site): PKH67-labeled membrane patches and intracellular vesicles were found in DC, and CD3 surface-labeling stained not only T lymphocytes, but also patches on DC. Similar patterns were obtained when H9-HIV cells were labeled with carboxyfluorescein diacetate-succinimidyl ester (data not shown), a dye that labels cytoplasmic proteins, indicating that transfer is not restricted to plasma membrane-associated material. Equivalent results were obtained by using live HIV_{lai}-infected primary CD4+ T cell blasts: PKH67-labeled material was found in 26 ± 6% of DC at 20 min and 50 ± 14% at 2 h. Clear vesicular aspects were evidenced in half of the cells that had incorporated PKH67-stained material. A majority of PKH-positive vesicles colocalized with transferrin (64 ± 5%) or AcLDL (75 ± 7%, Fig. 4*E*). Therefore, live cell material was effectively internalized into DC and undergoes

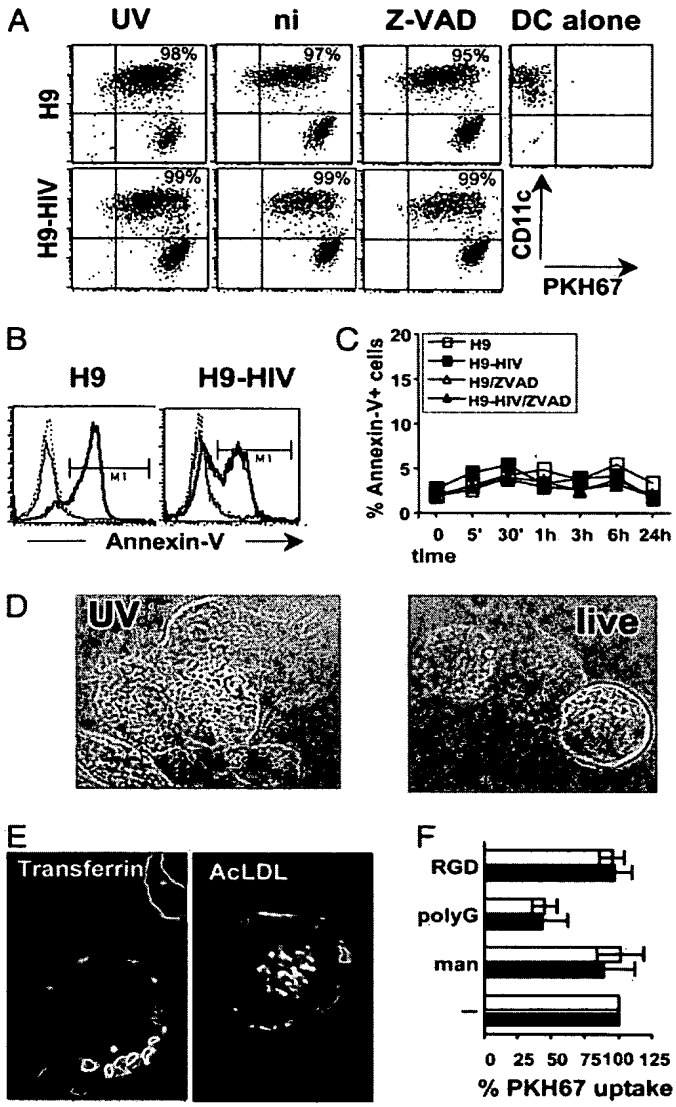

Fig. 4. Uptake of cell material from HIV-infected live cells by DC. (*A*) CD11c and PKH67 labeling of DC gated using forward scatter (FSC) and side scatter (SSC) criteria (note: some H9 cells appear in this gate as CD11c-negative events) after overnight coculture with PKH67-labeled H9 cells at an H9:DC ratio of 3:1. Dot plots show DC that have acquired H9-derived material as $CD11c^+$ $PHK67^+$ events, and their percentages are noted. UV, UV-irradiated; ni, nonirradiated; Z-VAD, nonirradiated Z-VAD-treated H9 cells. (*B*) Annexin-V staining of H9 cells gated using FSC and SSC criteria and $CD11c^-$, $PKH67^+$ labeling after overnight incubation alone (dotted lines), or with DC (thick line, UV-irradiated; thin line, nonirradiated H9 cells). (*C*) Annexin-V staining of H9 cells after different incubation times with DC. (*D*) Microscopy visualization of DC interaction with H9-HIV cells. Transmission light and confocal fluorescence overlay. DC were cultured for 15 min with UV-irradiated (UV) or nonirradiated (live) H9-HIV cells, treated with Z-VAD. They were fixed, permeabilized, and labeled for CD3 (green) and TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling) reaction (red) to detect apoptotic cells. Intimate membrane interactions were found between DC (unlabeled) and H9-HIV cells (green). (*E*) Material from live, infected, PKH26-labeled (red) $CD4^+$ T cell blasts is internalized and colocalizes (in yellow) with transferrin or AcLDL after 2 h of incubation with DC. (*F*) Competition of live (filled) or UV-irradiated (open) H9-HIV-associated (positive for CD11c, in blue) PKH67 uptake by DC using several adhesion molecule ligands. man, mannan. Mean values and SDs of at least three independent experiments are represented. DC were 60–95% PKH67-positive in absence of competitors. Data are representative of at least three experiments, except for *C* (two experiments).

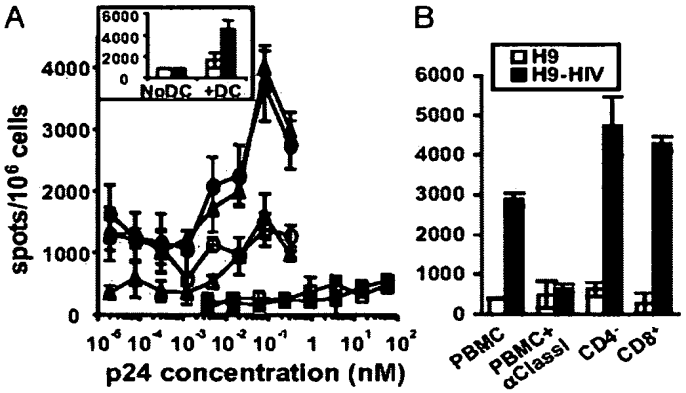

Fig. 5. Cross-presentation of HIV antigens from live infected cells. (*A*) DC were tested as in Fig. 3 after coculture with UV-irradiated (circles) or live, Z-VAD-treated (triangles) H9 (empty symbols) or H9-HIV (filled symbols) cells in the presence of AZT, by using an anti-RT_{476-84} $CD8^+$ line (DC:T ratio 1:1), all in the presence of LPS. DC were also incubated with free viral particles in the presence (open squares) or absence (filled squares) of AZT as in Fig. 3. (*Inset*) Live H9 (open bars) and H9-HIV (filled bars) cells were incubated or not with DC in the presence of Z-VAD and tested as before. (*B*) Recognition of DC cocultured with Z-VAD-treated H9-HIV cells by circulating lymphocytes from an HIV^+ patient (DC:T ratio 1:5) in the presence of LPS and AZT. PBMC were incubated with an anti-class I ascitis, CD4-depleted or CD8-enriched. Data are representative of two experiments.

classical trafficking from early (transferrin-positive) to late endosomes (positive for AcLDL).

Several receptors have been implicated in apoptotic or live cell material uptake by DC (23–25). Both live and apoptotic cell-associated material uptake were inhibited by polyG and fucoidan, and not by mannan, Arg-Gly-Asp (RGD), or Arg-Gly-Asp-Ser (RGDS) (Fig. 4*F* and not shown), indicating uptake mediated by scavenger receptors and not mannose receptors or α integrins.

To determine whether this transfer from live cells led to HIV antigen presentation, DC were cultured overnight with irradiated or live Z-VAD-treated H9-HIV cells. Amazingly, the HIV-specific $CD8^+$ cell line recognized live infected H9 cells with a dose–response curve entirely superimposed to that of apoptotic cells (Fig. 5*A*). Similar results were obtained with Z-VAD untreated cells (data not shown). Live H9-HIV cells were not recognized in the absence of DC (Fig. 5*A Inset*). When DC-T contact was blocked by using a 0.45-μm transwell, cross-presentation was prevented (Fig. 8*A*, which is published as supporting information on the PNAS web site), despite passage of particles with a size similar to that of 100- to 430-nm beads (Fig. 8*B*). Consistently with previous results, a three-log excess of free virus over the maximal viral protein concentration in infected cells was unable to induce a detectable $CD8^+$ T cell stimulation (Fig. 5*A*). These data indicate that HIV antigen can be transferred from both apoptotic and live cells to DC, and that both mechanisms are equally efficient for MHC class I-restricted presentation. Moreover, cross-presented HIV antigens from live infected cells were recognized by circulating $CD8^+$ T cells isolated from HIV-infected patients (Fig. 5*B*), adding more *in vivo* relevance to these results.

Discussion

The data presented here show that antigens from not only apoptotic, but also live, infected $CD4^+$ T cells can be presented by DC much more efficiently than live virus or replication-deficient viral particles by using quantitative tests. This cross-presentation was restricted by DC MHC class I molecules, occurred in the absence of viral replication or viral particles, and

required contact between DC and infected T cells. Antigen transfer from nonirradiated $CD4^+$ lymphocytes to DC and subsequent cross-presentation occurred from live T cells and not from contaminating apoptotic cells, either present as a minor population in the culture or secondarily induced by HIV-exposed DC. Indeed, it was not prevented by a pan-caspase inhibitor because in these conditions apoptosis was not induced by DC anytime during coculture, and the dose–response curves obtained with irradiated and nonirradiated $CD4^+$-infected cells were superimposable. Therefore, we demonstrate cross-presentation of infectious antigens from live antigen-donor cells as in an elegant model using OVA-expressing recombinant vaccinia viruses (26). We questioned the mechanism of antigen acquisition from live infected cells and the potential relevance of these different presentation mechanisms for HIV infection.

Different mechanisms for antigen exchange between T lymphocytes and DC are possible: exosome (27) or microparticle transfer (28), or antigen uptake from whole cells (29, 30). Antigen cross-presentation was blocked by a 0.45-nm membrane, despite demonstrated passage of particles with sizes compatible with that of exosomes (50–90 nm), or microvesicles generated by H9-HIV cells (50–500 nm) (31). If these particles were the major source of HIV antigens for DC cross-presentation, then their transfer would not need close DC:T cell contact, as those found by microscopy. The nibbling mechanism, implying cell-associated material exchange, has been described for antigen transfer from DC, macrophages, B cells, and activated T lymphocytes to monkey DC, allowing cross-presentation of a tumor antigen to $CD8^+$ lymphocytes (30). Here, capture of both live and apoptotic cell material seemed dependent on scavenger receptors, and not on mannose-binding lectins or integrins. Redundant pathways difficult to inhibit by a single antibody may be involved. This process is distinct from the reverse acquisition of membrane material from antigen-presenting cells by T cells, which occurs after immunological synapse formation, only during cognate interaction between the T cell receptor and epitope-loaded MHC molecules (32, 33). Interaction between DC and T lymphocytes has already been reported in the absence of cognate antigen or relevant MHC expression in DC (34). This interaction, characterized as an antigen-independent synapse, induces signaling in T lymphocytes, as well as in DC (35) that may stimulate nibbling. HIV antigens may be recruited at the site of the synapse, as occurs in interactions between HIV-infected DC and T cell lines (36).

The different MHC class I-restricted presentation pathways that have been described for HIV antigens in DC, i.e., classical presentation after direct infection and cross-presentation after defective virus entry (13) or after phagocytosis of infected apoptotic $CD4^+$ T lymphocytes (11, 12), had never been compared quantitatively. Our experiments attempted to reproduce *in vivo* conditions by using $CD8^+$ T cell lines with a relative low avidity or even $CD8^+$ cells purified from HIV^+ patient PBMC. We also validated the results obtained with chronically infected immortalized $CD4^+$ T cells using primary $CD4^+$ T lymphocytes together with a primary viral isolate. The role of apoptosis in enhancing cross-presentation has up to now been extensively compared with that of necrosis (10, 37), but not quantitatively with that of live cell-associated antigen transfer. The equivalent efficacy of apoptotic or live cell cross-presentation in the present study may be coincidental because the two pathways presumably use different and redundant receptors to internalize antigens into DC (23, 24).

We show that apoptotic or live cell cross-presentation needs very low amounts of HIV proteins to reach the threshold for efficient $CD8^+$ T cell stimulation. Conversely, HIV epitope presentation after direct infection of DC was not detectable, even with high amounts of replicative virus. Efficient presentation after DC infection by live or defective virus probably needs recognition by high-avidity $CD8^+$ T clones (13) whereas it was not found in another study using PBMC from patients (11). Therefore, *in vivo*, cross-presentation should allow recognition of cells expressing very low amounts of viral proteins by naive or average-avidity memory $CD8^+$ T lymphocytes.

Cross-presentation can induce immunity or tolerance, depending on the environment and the activation state of the DC. In the absence of CD40-CD40L costimulation, it leads to tolerance or even suppression of immune responses (10, 38, 39). A striking correlation was shown between the frequency of cytotoxic T lymphocytes specific for vinculin, an antigen overexpressed in apoptotic cells, and the proportion of peripheral apoptotic $CD40L^+$ T cells in HIV-infected patients, implying that vinculin is cross-presented by DC from $CD40L^+$ T cells (22). In the present study, the apparent lack of requirement for DC maturation stimuli may be related to the use of HIV-specific $CD8^+$ T cell lines, which need less costimulation than PBMC. It may also be related to the HIV- and apoptosis-independent induction of DC maturation by primary $CD4^+$ T cell blasts. In our hands, the proportion of $CD83^+$ (mature) cells (Fig. 6) and CD40 expression (not shown) increased when DC were incubated with primary $CD4^+$ T cell blasts, whether these blasts were apoptotic or not, independently of HIV infection. In former studies, when PBMC from HIV-infected patients and not T cell lines were used, soluble CD40L or $CD4^+$ T cell help or LPS were indeed required (11). From all these data, and because HIV infects predominantly activated lymphocytes (40), it is likely that, *in vivo*, live and apoptotic HIV-infected T lymphocytes can supply antigens and costimulation signals for MHC class I-restricted presentation by DC and thus immunostimulation. On the other hand, they may induce tolerance, depending on HIV infection stage, because, in HIV patients with low CD4 counts, triggering of CD40L on T cells is impaired (41). Finally, the outcome of live infected cell cross-presentation might be stimulation or tolerance and needs to be further explored.

In vivo, the infection route was shown to influence the nature of the pathway used for MHC class I-restricted presentation (42). This pathway may depend on the cell types encountered by the antigens. DC are required for $CD8^+$ lymphocyte cross-priming *in vivo*, even after infection with bacteria infecting macrophages (5). Cross priming after HSV-1 s.c. infection occurs rapidly in lymph nodes in the absence of local virus, suggesting rapid and efficient antigen uptake and presentation by DC (43). This activity requires viral protein synthesis, indicating that DC capture antigens from productively infected cells, but the model did not discriminate whether donor-infected cells were alive or apoptotic. Cross-presentation of live cell-associated antigens might allow the development of an early immune response at the first stages of HIV infection, before apoptosis is massively induced. Moreover, HIV antigen acquisition from live cells by DC could be an efficient mechanism to induce recognition of very low amounts of viral proteins and destruction of latent viral reservoirs. This mechanism could probably be enhanced by using either granulocyte colony-stimulating factor to promote limited viral production in resting T lymphocytes and macrophages (44, 45), or IL-2 to promote viral production in T cells and restore T cell help (46, 47), combined with IFNα to enhance cross-presentation, and $CD4^+$ T helper 1 and $CD8^+$ T cell effector functions (48, 49), during highly active antiretroviral treatment-structured interruptions. Thus, eradication of reservoirs might be obtained.

We acknowledge R. Thomas, A. Trautmann, and R. Cheynier for critically reading the manuscript, S. Wain Hobson for interesting discussion, A. Benmerah for transferrin and valuable advice, A. C. Ripoche for technical help, A. Jobard and the Cochin Institute confocal platform for teaching confocal microscopy, M. Lichtner and M. Andreoni (Sapienza University, Rome) for WT virus, and P. Langlade (Pasteur

Institute, Paris) for W6/32 ascitis. This work was supported by grants from Agence Nationale de Recherche sur le SIDA (ANRS), Ensemble Contre le SIDA (ECS), and fellowships from Institut National de la Santé et de la Recherche Médicale (to C.M.), the European Community (QLK2-2000-52160, to C.M.), Ministère de la Recherche et de la Technologie (to W.C.), and ECS (J.-F.D.).

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The opinion in support of the decision being entered today is *not* binding precedent of the Board.

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte
LOUIS D. FALO, JR. and CHRISTINA M. CELLUZZI

Appeal 2007-1029[1]
Application 09/208,549
Technology Center 1600

DECIDED: July 12, 2007

Before TONI R. SCHEINER, DONALD E. ADAMS, and RICHARD M. LEBOVITZ, *Administrative Patent Judges*.

SCHEINER, *Administrative Patent Judge*.

DECISION ON APPEAL

This appeal under 35 U.S.C. § 134 involves claims 1-3 and 5-12, directed to a formulation comprising hybridomas of tumor cells fused to dendritic cells or macrophages. The claims stand rejected as anticipated by, and as obvious over the prior art. We have jurisdiction under 35 U.S.C. § 6(b). We affirm.

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[1] Heard May 17, 2007.

BACKGROUND

> T-cells, including cytotoxic T-lymphocytes (CTLs), are a critical component of effective human immune responses to tumors and viral infections . . . CTLs destroy neoplastic cells or virus infected cells through recognition of antigenic peptides presented by MHC Class I molecules on the surface of the effected target cells. These antigenic peptides are degradation products of foreign proteins present in the cytosol of the effected cell[s], which are processed and presented to CTLs through the endogenous MHC Class I processing pathway. CTLs target tumors through recognition of a ligand consisting of a self MHC Class I molecule and a peptide antigen. The development of CTL-dependent anti-tumor immunization strategies, therefore, typically depends on both the identification of tumor antigens recognized by CTLs and the development of methods for effective antigen delivery.
>
> Although the recognition of a foreign protein in the context of the MHC Class I molecule may be sufficient for the recognition and destruction of effected target cells by CTLs, the induction of antigen-specific CTLs from T-lymphocyte precursors requires additional signals. Specialized antigen-presenting cells (APCs) can provide both the antigen MHC Class I ligand and the accessory signals required in the induction phase of CTL mediated immunity . . . APCs include, for example, macrophages, B-cells, and dendritic cells . . . .

Spec. 1: 12 to 2: 12.

> Tumor cells . . . express antigens which can be targeted by CTLs, but the tumor cells . . . themselves do not stimulate CTL immunity. This is presumably because the tumor cells . . . are incapable of providing the antigen or antigens in the appropriate context of co-stimulation. Antigen presenting cells (APC), however, express a variety of co-stimulatory molecules and cytokines . . .

Id. at 4: 5-10.

DISCUSSION

The present invention "provides a formulation comprising one or more hybridomas comprised of an antigen presenting cell fused to a tumor cell" (*id.* at 3: 21-22). "The fused cells . . . are then used to provide a complete array of tumor antigens . . . that can be delivered to the endogenous pathway of APCs from MHC Class I specific presentation and CTL stimulation. Fusion . . . of the APCs with the tumor cells . . . causes the tumor cells to become more immunogenic by association with the professional APCs. The fusion products . . . express properties of both the APC and the tumor; these products are capable of priming a CTL response. This results in the destruction of tumor cells that express similar tumor antigens" (*id.* at 4: 12-20).

Claims 1 and 3 are representative of the subject matter on appeal:

1. A formulation comprising at least one hybridoma having at least one first cell fused to at least one second cell, wherein said first cell is an antigen presenting cell selected from the group consisting of a macrophage and a dendritic cell, wherein said second cell is a tumor cell, and wherein said dendritic cell induces effective CTL-dependent anti-tumor immunity.

3. The formulation of claim 1, wherein said tumor cells are selected from the group consisting of melanoma cells, lung carcinoma cells, sarcomas, prostate carcinoma cells, breast carcinoma cells, colon carcinoma cells and cervical carcinoma cells.

DISCUSSION

The Examiner rejected claims 1-3 under 35 U.S.C. § 102(b) as anticipated by Peters.[2] In addition, the Examiner rejected claims 1, 2, and 5-12 under 35 U.S.C. § 103 as unpatentable over Guo[3] and Sornasse,[4] and claim 3 as unpatentable over Guo, Sornasse, and the Merck Manual.[5]

Appellants do not argue the claims separately. Therefore, the claims subject to each rejection will stand or fall together, as provided for in 37 C.F.R. § 41.37(c)(1)(vii). We select claims 1 and 3 as representative of the subject matter on appeal.

Claims 1-3, Anticipation

The Examiner rejected claims 1-3 under 35 U.S.C. § 102(b), relying on Peters as evidence of anticipation.

Peters describes "a number of DC [dendritic cell] hybridomas . . . which retained selected and combined [dendritic cell] properties" (Peters at 159). "[M]ouse [dendritic cells] were prepared . . . [which] demonstrated little or no phagocytosis, were adherent to hydrophobic surfaces and weakly positive for unspecific esterase" (*id.*). They were fused with either lymphosarcoma cells or plasmocytoma cells to form the hybridomas. "In order to select for hybridomas approaching the [dendritic cell] phenotype,

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[2] J.H. Peters (Peters), *Dendritic Cell (DC) Hybridoma Action on T Lymphocyte Proliferation*, 7 Immunobiology 159 (1981).
[3] Yajun Guo et al., *Effective Tumor Vaccine Generated by Fusion of Hepatoma Cells with Activated B Cells*, 263 Science 518 (1994).
[4] Thierry Sornasse, *Antigen-Pulsed Dendritic Cells Can Efficiently Induce an Antibody Response In Vivo*, 175 J. Exp. Med. 15 (1992).
[5] THE MERCK MANUAL, 17th Ed., M.H. Beers and R. Berkow, eds., Merck Co., 1287 (1992).

the cells were kept on hydrophobic surfaces . . . and non-adherent cells were continuously eliminated" (*id.*). Fourteen different adherent lines were isolated which "differ[ed] markedly in their phenotype, both morphologically and functionally" (*id.*) "Three of the tested lines exhibit[ed] a strong inductive capacity on T lymphocyte growth[,]" and one of those three lines "stain[ed] positive with anti Ia serum" (*id.*).

The Examiner, relying at least in part on background information on page 2 of the Specification, asserts that induction of CTL-dependent immunity is "an inherent property of [dendritic cells]" (Answer 7).

"[I]n an *ex parte* proceeding to obtain a patent, . . . the Patent Office has the initial burden of coming forward with some sort of evidence tending to disprove novelty." *See In re Wilder*, 429 F.2d 447, 450, 166 USPQ 545, 548 (CCPA 1970). Nevertheless, "when the PTO shows *sound basis* for believing that the products of the applicant and the prior art are the same, the applicant has the burden of showing that they are not." *In re Spada*, 911 F.2d 705, 708, 15 USPQ2d 1655, 1658 (Fed. Cir. 1990) (emphasis added).

Given Peters' explicit identification of the cells used to make the hybridomas as dendritic cells, after due regard to their morphological and functional properties, we find that the Examiner has established a sound basis for believing that Peters' hybridomas are fusions between dendritic cells and tumor cells. Thus, the issue raised by this appeal is whether Appellants have met their burden of showing otherwise.

Appellants argue that "it would be surprising to one of skill in the art if Peters' cells were actually dendritic cells" (Br. 9), and Peters' "purported

dendritic cells . . . could have been any number of cells and were not necessarily dendritic cells" (*id.* at 8).

In particular, Appellants argue that "dendritic cells were neither well-defined in the art nor easily detectable when the Peters article was published" (*id.* at 7), citing Kuby,[6] and Karman[7] (erroneously identified as Ling), as evidence. Nevertheless, Appellants do not point to anything specific in either of these references, and it is not clear how the references support Appellants' position. Kuby teaches that "[d]endritic cells have been very difficult to study because conventional procedures for isolating lymphocytes and accessory immune-system cells tend to damage their long dendritic processes, so that the cells fail to survive" (Kuby at 69), but the issue with Peters is not one of isolating viable cells, but their identification once isolated. Similarly, Karman teaches that "the role of [dendritic] cells in central nervous system (CNS) immunity is unclear" and "[t]he diverse phenotypes and origins of [dendritic cells] make the characterization of their function in the [central nervous system] even more difficult" (Karman Abstract). Again, it is not clear how this has a bearing on Peters' identification of the isolated mouse cells as dendritic cells.

Along these same lines, Appellants argue that "other cell types, such as fibroblasts and endothelial cells . . . exhibit 'little or no phagocytosis,' are 'adherent to hydrophobic surfaces,' and are 'weakly positive for unspecific esterase[ ]'" (Br. 8). Nevertheless, Appellants have not identified any

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[6] Janis Kuby, IMMUNOLOGY, 3d ed., W.H. Freeman & Co., NY, 69 (1997).
[7] J. Karman et al., *Dendritic Cells in the Initiation of Immune Responses Against Central Nervous System-Derived Antigens*, 92 Immunol. Lett. 107, Abstract only (2004).

properties of Peters' cells that are inconsistent with Peters' description of the cells as dendritic cells. Moreover, even though there are morphological and functional differences among dendritic cells, and some dendritic cells might have some of the same properties as fibroblasts or endothelial cells, Appellants have not provided any evidence that one skilled in the art would have confused isolated fibroblasts or endothelial cells with isolated dendritic cells. Attorney argument is not evidence. *In re Pearson*, 494 F.2d 1399, 1405, 181 USPQ 641, 646 (CCPA 1974). Nor can it take the place of evidence lacking in the record. *Meitzner v. Mindick*, 549 F.2d 775, 782, 193 USPQ 17, 22 (CCPA 1977).

Finally, Appellants argue that "Peters does not provide any evidence . . . that the alleged dendritic cells 'induce[d] effective CTL-dependent anti-tumor immunity,' as required by the claims" (Br. 11).

However, as explained in the Specification, dendritic cells "can induce effective CTL-dependent anti-tumor immunity" (Spec. 2: 14-15), and Peters discloses that the dendritic cell hybridomas "retained selected and combined [dendritic cell] properties" (Peters at 159).

Again, we find that the Examiner has established a sound basis for believing that Peters' hybridomas were fusions between dendritic cells and tumor cells, and that the fused dendritic cells retained their intrinsic ability to induce CTL-dependent anti-tumor immunity, shifting the burden to Appellants to show otherwise. We further find that Appellants have not adequately discharged their burden of rebuttal, by argument or evidence.

Accordingly, we affirm the anticipation rejection claim of claim 1. As discussed above, claims 2 and 3 fall with claim 1.

Claims 1, 2, and 5-12, Obviousness

Claims 1, 2, and 5-12 stand rejected under 35 U.S.C. § 103(a) as unpatentable over Guo and Sornasse.

Guo teaches that

> Tumor cells may escape immune surveillance because they do not express signals that are essential for activation of the host immune system [.]. At the molecular level, the defective signaling of tumor cells could be attributable to (i) down-regulation of major histocompatibility complex (MHC) molecules [ ]; (ii) alteration of antigen-processing pathways, resulting in an inability to present tumor-specific antigens to host T cells [ ]; (iii) absence of costimulatory or adhesion molecules that are essential for activation of the host immune system [ ]; or (iv) production of factors that modify host immune responses [ ].

Guo at 518, left-hand col.

Guo fused rat hepatocellular carcinoma cells with activated B cells, "hypothesiz[ing] that fusion of a tumor cell with an activated B cell would produce a hybridoma that both expressed tumor-specific antigens and had the machinery for antigen presentation" (Gou at 518, left-hand col.). The parental tumor cells expressed low levels of MHC class I antigens and intracellular adhesion molecule-1 (ICAM-1), but lacked MHC class II antigen, leukocyte functional antigen-1 (LFA-1), and B7. In contrast, the hybridoma cell lines expressed MHC class II antigens, ICAM-1, LFA-1, and B7, and stably expressed both tumor and B cell antigens (*id.* at 518, right-hand col.). The hybridomas lost the tumorigenicity of the parent hepatocellular carcinoma cells, and became immunogenic. Rats injected

with the hybridomas became resistant to challenge with the parent tumor cells, and rats with established hepatocellular carcinomas were cured by subsequent injection of the hybridomas (*id.* at 519, left and center col.).

Sornasse teaches that dendritic cells, "which most efficiently activate a primary T cell response in vitro" (Sornasse at 15, left-hand col.), "have some unique properties in vivo, as compared with the other [antigen presenting cells]" (*id.*). For example, dendritic cells "seem to be the major source of processed antigen in vivo" and "appear to play a major role in initiating various T cell immune responses in vivo, such as . . . activation of MHC-restricted T cell responses" (*id.*). Fresh dendritic cells pulsed with intact myoglobin were able "to very efficiently generate the antigenic epitopes [ ] that can be presented by self-MHC" (*id.* at 17, right-hand col.), and were able to generate a strong specific B cell response in vivo in unprimed animals subsequently boosted with soluble antigen. Low density B cells pulsed with myoglobin "very efficiently present[ed] myoglobin . . . in vitro" (*id.* at 18, left-hand col.), but "only induce[d] a weak primary response in vivo as compared with [dendritic cells]' (*id.*). Finally, Sornasse "emphasize[s] the main role of [dendritic cells] in initiating primary responses in vivo" (*id.* at 18, left-hand col.), and suggests that dendritic cells may be used "to induce cellular [ ] and T cell-dependent humoral responses in vivo" (*id.* at 19, right-hand col.).

According to the Examiner, "[i]t would have been *prima facie* obvious to one of ordinary skill in the art . . . to produce a plurality of hybrids (or hybridomas) . . . as taught by Guo . . . substituting a [dendritic cell] for the B cell in said hybrid" (Answer 5), because Sornasse

"emphasize[s] the main role of [dendritic cells] in initiating primary responses in vivo" and teaches that "both B cells and [dendritic cells] are capable of inducing IL2 secretion *in vitro*, [but] [dendritic cells] induce a more vigorous response, including a Th1 response, *in vivo*" (*id.*).

Appellants argue that "there is no suggestion to substitute a dendritic cell for the B cell of Guo" (Br. 12), because "Guo teaches away from the use of a dendritic cell by stating that B cells are the most effective antigen presenting cells" (*id.* at 13), and "Sornasse supports this teaching by stating that dendritic cells presented antigen poorly compared to B cells" (*id.* at 12, citing Sornasse at 16, right hand col., last sentence of the first paragraph).

First of all, we are not persuaded by Appellants' assertion that Guo teaches away from the use of dendritic cells by stating that B cells are the most effective antigen presenting cells.[8] A reference is said to "teach away" from a claimed invention when it "suggests that the line of development flowing from the reference's disclosure is unlikely to be productive of the result sought by the applicant" (*In re Gurley*, 27 F.3d 551, 553, 31 USPQ2d 1130, 1131 (Fed. Cir. 1994)). That is not the case here. Guo makes no mention of dendritic cells, thus, there is nothing in Guo's disclosure that suggests that substituting a dendritic cell for a B cell in Guo's hybridomas would be unlikely to be productive.

Nor are we persuaded by Appellants' argument regarding Sornasse, as it depends on a teaching taken completely out of context. Appellants assert

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[8] For the sake of accuracy, we note that Guo actually states that "*[a]ctivated* B cells are the most effective antigen-presenting cells" (Guo at 518, emphasis added).

that Sornasse "states that 'DC cultured with antigen only slightly induce the activation of the T cell hybridoma, whereas . . . B cells very efficiently present the antigen in the same condition'" (Br. 13). But that selective excerpt from Sornasse is only part of the paragraph bridging columns 1 and 2 of page 16, which reads as follows:

> *Fresh Splenic Dendritic Cells Present Native Proteins In Vitro.* The initiation of any T cell response requires two independent steps: Ag presentation and T cell sensitization. Ag presentation generates the ligand that is recognized by the . . . clonally specific portion of the TCR for Ag, and usually requires the generation of small peptides. Although it is clear that purified splenic DC have a poor, if any, capacity for processing [native proteins], data . . . clearly showed that handling of intact proteins was down-regulated in cultured as compared with fresh epidermal [dendritic] cells . . . [and] that fresh splenic DC were able to process native proteins early, i.e., during the purification procedure of the DC. In a preliminary experiment, we compared the ability of dendritic cells and control cells to process and present myoglobin in vitro. We used a T cell hybridoma, since its activation only requires TCR occupancy, i.e., the presence of the appropriate antigen in the context of self MHC, and does not depend on any costimulatory signal [ ]. As control APC we chose cells that were isolated from the same low density fraction as the dendritic cells, but were nonadherent during the 2-h culture and were depleted of T cells . . . [B]oth APC populations (DC or low-density B cells), pulsed during overnight culture, strongly induce IL-2 secretion by a myoglobin-specifc T cell hybridoma. The two types of APC, however, have distinct properties. Indeed, 24 h-old, purified DC cultured with antigen only slightly induce the activation of the T cell hybridoma, whereas 24 h-old low-density B cells very efficiently present the antigen in the same conditions.

Thus, as summarized by Sornasse, "the capacity of the DC population to process and present proteins was downregulated when the dendritic cells matured in culture" (Sornasse at 15, right-hand col.), but fresh dendritic cells pulsed with native protein were able "to very efficiently generate the antigenic epitopes [ ] that can be presented by self-MHC" (*id.* at 17, right-hand col.). Again, Sornasse's data "emphasize the main role of [dendritic cells] in initiating primary responses in vivo" (*id.* at 18, left-hand col.).

We find that the Examiner has established a prima facie case that it would have been obvious for one of ordinary skill in the art to substitute dendritic cells for Guo's activated B cells in Guo's hybridomas, which Appellants have not overcome by argument or evidence. Accordingly, the rejection of claims 1, 2, and 5-12 under 35 U.S.C. § 103(a) as unpatentable over Guo and Sornasse is affirmed.

Claim 3, Obviousness

In addition, claim 3 stands rejected under 35 U.S.C. § 103(a) as unpatentable over Guo, Sornasse, and the Merck Manual.

Neither Guo nor Sornasse teaches a formulation that includes tumor cells or antigens of the particular types recited in claim 3. The Examiner argues that it would have been obvious for one of ordinary skill in the art to use melanoma cells in dendritic cell hybridomas, in order to "produce a formulation for inducing effective CTL immunity against melanoma" (Answer 6), "given the teachings of the Merck Manual that conventional treatments offer no proven benefit for melanoma" (*id.*).

Appellants argue only that "The Merck Manual does not remedy the deficiencies of Guo and Sornasse" (Br. 15). However, as discussed above,

we disagree with Appellants' assertions of deficiencies in the Examiner's proposed combination of Guo and Sornasse. Accordingly, the rejection of claim 3 under 35 U.S.C. § 103(a) is affirmed.

SUMMARY

The Examiner has established a sound basis for believing that Peters describes dendritic cell hybridomas, which Appellants have not adequately rebutted by argument or evidence. Thus, we affirm the anticipation rejection of claims 1-3. The Examiner has also established a prima facie case that the presently claimed subject matter would have been obvious over Guo, Sornasse, and The Merck Manual, thus we affirm the obviousness rejection of claims 1, 2, and 5-12, as well as the obviousness rejection of claim 3.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a).

AFFIRMED

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APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
09/208,549	12/09/1998	LOUIS D. FALO, JR.	214001-00705

CONFIRMATION NO. 8235

OC000000006145296

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NOTICE REGARDING POWER OF ATTORNEY

This is in response to the Power of Attorney filed 05/29/2001.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

Joan Macewood

Customer Service Center
Initial Patent Examination Division (703) 308-1202

ATTORNEY/APPLICANT COPY

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I HEREBY DECLARE:

THAT my residence, post office address, and citizenship are as stated below next to my name;

THAT I believe I am the original, first, and sole inventor (if only one inventor is named below) or an original, first, and joint inventor (if plural inventors are named below or in an attached Declaration) of the subject matter which is claimed and for which a patent is sought on the invention entitled

INDUCTION OF TUMOR AND VIRAL IMMUNITY USING ANTIGEN PRESENTING CELL CO-CULTURE PRODUCTS AND FUSION PRODUCTS

(Attorney Docket No. 076333-0242)

the specification of which (check one)

___ Is attached hereto.

__X__ Was filed on December 9, 1998 as United States Application Number or PCT International Application Number 09/208,549 and was amended on _____ (if applicable).

THAT I do not know and do not believe that the same invention was ever known or used by others in the United States of America, or was patented or described in any printed publication in any country, before I (we) invented it;

THAT I do not know and do not believe that the same invention was patented or described in any printed publication in any country, or in public use or on sale in the United States of America, for more than one year prior to the filing date of this United States application;

THAT I do not know and do not believe that the same invention was first patented or made the subject of an inventor's certificate that issued in any country foreign to the United States of America before the filing date of this United States application if the foreign application was filed by me (us), or by my (our) legal representatives or assigns, more than twelve months (six months for design patents) prior to the filing date of this United States application;

THAT I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment specifically referred to above;

THAT I believe that the above-identified specification contains a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention, and sets forth the best mode contemplated by me of carrying out the invention; and

THAT I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I HEREBY CLAIM foreign priority benefits under Title 35, United States Code §119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number	Country	Foreign Filing Date	Priority Claimed?	Certified Copy Attached?

I HEREBY CLAIM the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

U.S. Provisional Application Number	Filing Date
60/039,472	2/27/97

I HEREBY CLAIM the benefit under Title 35, United States Code, §120 of any United States application(s), or § 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application Number	PCT Parent Application Number	Parent Filing Date	Parent Patent Number
09/030,985		02/26/98	

I HEREBY APPOINT the following registered attorneys and agents of the law firm of FOLEY & LARDNER:

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to have full power to prosecute this application and any continuations, divisions, reissues, and reexaminations thereof, to receive the patent, and to transact all business in the United States Patent and Trademark Office connected therewith.

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I UNDERSTAND AND AGREE THAT the foregoing attorneys and agents appointed by me to prosecute this application do not personally represent me or my legal interests, but instead represent the interests of the legal owner(s) of the invention described in this application.

I FURTHER DECLARE THAT all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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Inventor's signature	
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